



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

011273

OCT 6 1994

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

**MEMORANDUM:**

**SUBJECT:** 2,4-D: Review of acute neurotoxicity screening battery  
(§81-8)

**EPA IDENTIFICATION NOS.:** MRID No.: 431152-01  
Pesticide Chemical Code: 030081  
Toxicology Chemical Code: 315  
DP Barcode: D200086  
Submission No.: S459751

**FROM:**

Robert F. Fricke, Ph.D. *Robert F. Fricke 4 OCT 94*  
Toxicology Branch II, Section IV  
Health Effects Division (7509C)

**TO:**

Walter Waldrop  
Product Manager (71)  
Registration Division (7505C)

**THRU:**

Susan L. Makris *Susan L. Makris 10/4/94*  
Toxicology Branch II, Head Section IV  
Health Effects Division (7509C)

and

Marcia van Gemert, Ph.D. *Marcia van Gemert 10/5/94*  
Chief, Toxicology Branch II  
Health Effects Division (7509C)

**Registrant:** Industry Task Force II on 2,4-D Research Data

**Chemical:** 2,4-Dichlorophenoxyacetic acid, 2,4-D

**Action Requested:** Review Acute Neurotoxicity Screening Battery  
(§81-8) toxicology study in the rat to support reregistration.

1. In this acute neurotoxicity study, Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D at doses of 0 (corn oil), 15, 75, or 250 mg/kg (actual: 0, 13, 67 or 227 mg/kg). Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at



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Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized and neuropathological examination performed on control and treated animals (5/sex/dose).

No treatment-related mortalities occurred during the study.

No significant differences were noted in the mean body weights or mean body weight gains.

Clinical signs and neurobehavioral evaluation revealed treatment-related changes. During the Day 1 FOB evaluations, increased incidences of incoordination (6/10, males; 4/10, females) and slight gait abnormalities, described as forepaw flexing or knuckling, were observed in high-dose animals (8/10, males; 8/10 females). Slight gait abnormalities, observed in a single mid-dose female, were not judged to be treatment-related since no other signs of toxicity were evident. Minimal gait abnormalities, not judged to be treatment-related, were observed in one low-dose female and one each mid- and high-dose male. On the Day 2 and 3 clinical examinations, incoordination was noted in high-dose animals. The incidence of incoordination decreased to control levels by Day 4 in males and Day 5 in females. In high-dose animals total motor activity was significantly lower at Day 1 only.

No treatment-related gross or neuropathological findings were present.

Based on the results of this study, the LOEL for systemic toxicity was not determined; the NOEL for systemic toxicity was 227 mg/kg in males and females. The LOEL for neurobehavioral effects was 227 mg/kg in males and females; the NOEL for neurobehavioral effects was 67 mg/kg in males and females.

2. Conclusions: This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.



Reviewed by: Robert F. Fricke, Ph.D.  
Section IV, Tox. Branch II (7509C)

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Secondary Reviewer: Susan L. Makris, M.S.  
Section IV, Tox. Branch II (7509C) *Susan L. Makris 10/4/94*

#### DATA EVALUATION RECORD

**STUDY TYPE:** Acute Neurotoxicity Screening Battery - Rat (81-8)

**EPA ID NOS.:** MRID No.: 431152-01  
Pesticide Chemical Code: 030001  
Toxicology Chemical Code: 315  
DP Barcode: D200086  
Submission No.: S459751

**TEST MATERIAL:** 2,4-Dichlorophenoxyacetic acid

**SYNONYMS:** 2,4-D

**STUDY NUMBERS:** K-002372-066

**SPONSOR:** Industry Task Force II on 2,4-D Research Data

**TESTING LAB:** The Toxicology Research Laboratory  
Health and Environmental Sciences  
The Dow Chemical Company, Midland, MI

**REPORT TITLE:** 2,4-Dichlorophenoxyacetic Acid (2,4-D): Acute  
Neurotoxicity Study in Fischer 344 Rats

**AUTHORS:** J.L. Mattsson, R.J. McGuirk and B.L. Yano

**REPORT ISSUED:** 5 January 1994

**EXECUTIVE SUMMARY:** In this acute neurotoxicity study, Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D at doses of 0 (corn oil), 15, 75, or 250 mg/kg (actual: 0, 13, 67 or 227 mg/kg). Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized and neuropathological examination performed on control and treated animals (5/sex/dose).

No treatment-related mortalities occurred during the study.

No significant differences were noted in the mean body weights or mean body weight gains.

Clinical signs and neurobehavioral evaluation revealed treatment-related changes. During the Day 1 FOB evaluations, increased incidences of incoordination (6/10, males; 4/10, females) and slight gait abnormalities, described as forepaw flexing or knuckling, were observed in high-dose animals (8/10, males; 8/10 females). Slight gait abnormalities, observed in a single mid-

dose female, were not judged to be treatment-related since no other signs of toxicity were evident. Minimal gait abnormalities, not judged to be treatment-related, were observed in one low-dose female and one each mid- and high-dose male. On the Day 2 and 3 clinical examinations, incoordination was noted in high-dose animals. The incidence of incoordination decreased to control levels by Day 4 in males and Day 5 in females. In high-dose animals total motor activity was significantly lower at Day 1 only.

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Based on the results of this study, the LOEL for systemic toxicity was not determined in males and females; the NOEL for systemic toxicity was 227 mg/kg in males and females. The LOEL for neurobehavioral effects was 227 mg/kg in males and females; the NOEL for neurobehavioral effects was 67 mg/kg in males and females.

This study is classified as Core - Guideline and satisfies guideline requirements (§81-8) for an acute neurotoxicity screening battery in the rat.



## I. MATERIALS

A. Test Compound: 2,4-D, technical Description: white solid Lot No.: 909 (air-milled) Purity: 96.6%  
Contaminants: Not given

B. Test Animals: Species: Rat Strain: Fischer 344, Age: 8 weeks Weight (g): 132.2 - 155.5 (males), 95.3 - 118.9 (females) Source: Charles River Laboratories, Kingston, NY Housing: Individually in mesh-bottom cages Feed: Certified Purina Rodent Chow #5002 (Purina Mills, Inc., St. Louis, MO), ad libitum Water: tap water, ad libitum Environment: Temperature,  $22.2 \pm 0.3^{\circ}\text{C}$ ; Humidity,  $50.3 \pm 0.6\%$ ; Light/dark cycle, 12 hr/12 hr

## II. METHODS

A. Preliminary Study: A preliminary study was carried out to determine the time of peak effect and the highest non-lethal toxic dose. Animals (3/sex/dose) were orally gavaged with corn oil suspensions of 2,4-D at doses of 250, 500, 750 or 1000 mg/kg. Doses of 500 mg/kg or greater produced lethality. Toxicity, present in all treated animals, consisted of decreased muscle tone, increased lacrimation, fecal soiling (probably due to vehicle), incoordination, and knuckling of forepaws. To further refine the doses, a second preliminary study using 3 males/group was conducted at 0, 50, 100, 150 and 200 mg/kg dose levels. The benchmark dose was selected as 250 mg/kg based on increased salivation, incoordination and knuckling of forepaws. The time of peak effect occurred approximately 5 to 6 hours postdosing.

B. Study Design: Ten animals/sex/dose were randomly assigned to control and treatment groups (Table 1). Because of the complexity of study, the animals were further assigned to subgroups of 20 animals each; the subgroups were counter-balanced over the different doses and sexes. Each subgroup was stagger-started over a four day period. Following an overnight fast, animals were orally gavaged once with either corn oil (vehicle) or corn oil solution of 2,4-D followed by a 15-day observation period.

Table 1: Animal Assignment to Study Groups

Test Group	Dose Level* (mg/kg)	Number Assigned	
		Male	Female
Control	0	10	10
Low	15 (13)	10	10
Mid	75 (67)	10	10
High	250 (227)	10	10

\* Values in parentheses are the actual doses based on chemical analysis.



C. Dosing Preparations: A sufficient amount of 2,4-D was dissolved in corn oil to yield doses of 15, 75 and 250 mg/kg when administered in a dose volume of 10 ml/kg. Control animals received corn oil only. Dosing preparations were analyzed for stability and concentration.

D. Observations: Starting the day before exposure (Day -1) through terminal sacrifice, cageside observations were performed twice a day on all animals. On the days that the detailed clinical examinations (Days 2, 3, and 4) or behavioral testing (Days -1, 1, 8 and 15) were performed, the cageside observations were performed only once a day.

E. Body Weights: Body weights were determined on preexposure Day -1 and study Days 1, 2, 8, and 15.

F. Ophthalmological Examinations: Ophthalmological examinations were performed approximately two weeks prestudy and on Day 14.

G. Behavioral Tests: Behavioral tests consisted of the Functional Observational Battery (FOB) and evaluation of motor activity. Behavioral tests were performed Day -1, the day of dosing (Day 1), at the peak time of effect (approximately 5-6 hrs postdosing), and on Days 8 and 15.

1. Functional Observational Battery: The following parameters were evaluated:

Hand-held observations

General  
Palpebral closure  
Pupil size  
Lacrimation  
Salivation  
Skin/haircoat abnormalities  
Perineal staining  
Abnormal movements  
Convulsions, tremors  
muscle tone, etc.  
Abnormal respiration  
Reactivity to handling

Open-field observations

Level of activity  
Startle response  
Touch response  
Tail pinch response  
Gait abnormalities  
Abnormal/stereotypic behavior  
Quantity of urine/fecal pellets

Measurements/counts

Hindlimb grip strength  
Forelimb grip strength  
Landing foot splay

2. Motor activity: Motor activity was measured using automated photobeam activity recording devices; devices were calibrated before use each day. Motor activity was evaluated approximately five hours after dosing and consisted of six 8-minute epochs, totalling 48 minutes (asymptote at 30 to 40 minutes).

H. Pathological Examinations

1. Neuropathology: At Day 15, five animals/sex/dose were randomly selected for neuropathological examination. Heparinized animals were anesthetized and perfused transcardially, first with phosphate buffer containing 0.7% sodium nitrite, then with 1.5% glutaraldehyde/4% formaldehyde. The tissues collected for neuropathological examination are listed below.



### Tissues Selected for Neuropathological Examination

<u>Brain</u>	<u>Spinal Cord</u>
Olfactory lobe	Cervical & lumbar swelling
Cerebral cortex (frontal, parietal, temporal & occipital lobes)	<u>Peripheral Nerves</u>
Thalamus/hypothalamus	Sciatic, tibial, sural, caudal
Midbrain	<u>Skeletal Muscle</u>
Pons	Gastrocnemius, anterior tibial
Cerebellum	<u>Other</u>
Medulla oblongata	Pituitary gland
Nucleus gracilis/cuneatus	Trigeminal ganglia & nerve
<u>Dorsal Root Ganglia</u>	Eyes with optic nerves
Cervical & lumbar swelling	Nasal tissues with olfactory epithelium
<u>Dorsal &amp; Ventral Roots</u>	
Cervical & lumbar swelling	

2. Routine histopathology: Routine histopathological examination was performed on the remaining animals, following an overnight fast. The remaining animals were necropsied and the tissues listed below immersion fixed in neutral, phosphate-buffered 10% formalin.

### Tissues Selected for Routine Histopathology

<u>Digestive System</u>	<u>Cardiovascular/Hematology</u>	<u>Neurologic</u>
Tongue	Aorta	Brain (cerebrum, brainstem, cerebellum)
Salivary glands	Heart	Peripheral nerve
Esophagus	Bone marrow	Spinal cord (cervical, thoracic, lumbar)
Stomach	Mediastinal & mesenteric lymph nodes and tissue	Pituitary
Duodenum	Spleen	Eyes
Jejunum	Thymus	<u>Glandular</u>
Ileum	<u>Urogenital</u>	Adrenals
Cecum	Kidneys	Mammary gland
Colon	Urinary bladder	Parathyroids
Rectum	Testes	Thyroids
Liver	Epididymides	Auditory sebaceous glands
Pancreas	Prostate	Harderian/lacrimal glands
Oral tissues	Seminal vesicles	Coagulating glands
<u>Respiratory</u>	Ovaries	<u>Other</u>
Trachea	Uterus	Bone with joint
Lungs	Vagina	Skeletal muscle
Nasal tissues	Cervix	Skin and subcutis
Larynx	Oviducts	Gross lesions

H. Positive Controls: Positive control data were included in the study. For neurobehavioral evaluation, the following positive controls were used: Amphetamine (0.1, 0.32 or 1 mg/kg, ip) and chlorpromazine (0.5, 2.24 or 5 mg/kg, ip) for motor activity; amphetamine (8 mg/kg, ip), chlorpromazine (4 mg/kg, ip), or atropine (2 mg/kg, ip) plus physostigmine (0.75 mg/kg, sc) for FOB. Positive control neuropathology animals were treated with trimethyltin (7 mg/kg, po) on Day 1 followed by treatment with acrylamide (35 mg/kg, po) 5 times/week for 3 weeks. The positive control materials

accurately validated the FOB, motor activity and neuropathology findings.

**I. Statistical Evaluations:** The means and standard deviations of parametric data (body weight, hindlimb and forelimb grip strength, landing foot splay, and motor activity) were determined and variances (F-max test) evaluated for homogeneity. Grip strength was normalized for body weight; motor activity was expressed as the square roots of the counts. Repeated measures analyses (ANOVA or MANOVA) were used to evaluate different interactions (treatment  $\times$  time, treatment  $\times$  time  $\times$  sex, and/or treatment  $\times$  time  $\times$  epoch). The type I error rate ( $\alpha$ ) per comparison was set at 0.02 to decrease the rate of false positive responses.

### III. REGULATORY COMPLIANCES

A. Quality assurance was documented by signed and dated GLP and quality assurance statements.

B. A statement of "no confidentiality claims" was provided.

### IV. RESULTS

A. Analytical Chemistry: After 17 days, the low- mid- and high-dosing solutions were 93, 95 and 91% of the initial concentrations, respectively. The observed concentrations of the low-, mid- and high-dosing solutions were 13, 67 and 227 mg/kg, respectively.

B. Clinical Observations: On Day 1, one control and one high-dose female died as a result of gavage error; the high-dose female was replaced, while the control female was not. Treatment-related clinical signs were limited to high-dose animals, which showed uncoordinated movement/behavior (Table 2). On Day 2, 2/10 males and 5/10 females were affected. On Day 3, the incidence of affected males was unchanged at 2/10, while the number of affected females decreased to 1/10. A single female was affected on Day 4. Perineal soiling, noted in all dose groups, was attributed to the vehicle (corn oil).

Table 2: Clinical Signs (number affected/total number of animals)\*

Observation	Sex	Day	Dose Level (mg/kg)			
			0	15	75	250
Incoordination	Male	2	0/10	0/10	0/10	2/10
		3	0/10	0/10	0/10	2/10
		4	0/10	0/10	0/10	0/10
	Female	2	0/9	0/10	0/10	5/10
		3	0/9	0/10	0/10	1/10
		4	0/9	0/10	0/10	1/10

\* Data summarized from Table V-1 of the study.



C. Body Weights: No treatment-related differences in body weights were noted.

D. Ophthalmology: Ophthalmological examinations at Day 14 did not reveal any treatment-related effects.

E. Neurobehavioral Evaluations

1. FOB results: At the peak time of effect on Day 1, treatment-related FOB findings consisted of increased incidence of incoordination and gait abnormalities in high-dose animals; gait abnormalities were also observed in a single mid-dose female (Table 3). Gait abnormalities, graded as minimal, were not considered treatment-related because they were judged to be equivocal findings. Neither fore- and hindlimb grip strength nor landing foot splay were affected by treatment.

Table 3: FOB Findings (number affected/total number of animals)\*.

Observation	Sex	Day	Dose Level (mg/kg)			
			0	15	75	250
Incoordination	Male	-1	0/10	0/10	0/10	0/10
		1	0/10	0/10	0/10	6/10
		8	0/10	0/10	0/10	0/10
	Female	-1	0/9	0/10	0/10	0/9 <sup>b</sup>
		1	0/9	0/10	0/10	4/10
		8	0/9	0/10	0/10	0/10
Gait Abnormalities, (Grade = slight)	Male	-1	0/10	0/10	0/10	0/10
		1	0/10	0/10	0/10	8/10
		8	0/10	0/10	0/10	0/10
	Female	-1	0/9	0/10	0/10	0/9
		1	0/9	0/10	1/10	8/10
		8	0/9	0/10	0/10	0/10

\* Data summarized from Table V-2 of the study.

<sup>b</sup> One high-dose females died of gavage error and was immediately replaced. The replacement animal was not evaluated during the prestudy.

2. Motor activity results: On Day 1 at the peak time of effect, total motor activity was significantly decreased in high-dose animals.

Table 4: Total Motor Activity (square root of total beam breaks) Results\*

Sex	Day	Dose Level (mg/kg)			
		0	15	75	250
Male	-1	11.29	11.01	11.19	11.45
	1	7.50	8.61	8.46	4.89 <sup>a</sup>
	8	12.14	11.94	11.95	10.73
Female	-1	12.94	13.43	12.36	13.84
	1	9.12	8.98	9.64	6.28 <sup>a</sup>
	8	12.75	12.66	11.68	12.56

\* Data summarized from Table V-12 of the study.

<sup>a</sup> p = 0.015



#### F. Sacrifice and Pathology

1. Gross pathology: The incidence of gross pathological observations did not show any treatment-related effects.
2. Neuropathology: Microscopic evaluation of central and peripheral nervous system tissues did not reveal any treatment-related effects.

V. DISCUSSION and CONCLUSIONS: In this acute neurotoxicity study, Fischer 344 rats (10/sex/dose) were orally gavaged once with 2,4-D at doses of 0 (corn oil), 13, 67, or 227 mg/kg. Neurobehavioral evaluations, consisting of Functional Observational Battery (FOB) and motor activity, were conducted at Day -1 (prestudy), Day 1 (approximately 5-6 hrs postdosing, peak time of effect) and Days 8 and 15. At terminal sacrifice (Day 15), animals were euthanized; neuropathological examinations were performed on 5 animals/sex/dose.

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